REMARKS

This document is submitted in response to the Final Office Action mailed February 23, 2005 ("Office Action"). Claims 73-75, 88, 89, 98, and 99 have been canceled. Claims 24-27, 76, 90-91, and 100-101 have been amended and claims 103-112 added. Claims 1-23 and 28-72 were previously withdrawn from consideration.

Support for the amendments to the claims is as follows:

Claim 24

Claim 24 has been amended to clarify the class of bacterial cells encompassed, i.e., mutants of *Pseudomonas* strain AN5 having enhanced anti-fungal activity by virtue or producing higher levels of sugar acids on a cell basis than the parent strain. Support for claim 24 as now presented is to be found *inter alia* in original claim 24 and in the description at page 26, lines 4-10, teaching that mutants of *Pseudomonas* strain AN5 have "increased sugar acid biosynthetic capacity and/or increased sugar acid secretion compared to the naturally occurring isolate"; the description at page 66, lines 4-14, teaching that mutants of *Pseudomonas* strain AN5 producing more sugar acid also produce larger clearance zones on plate bioassays (i.e., have greater antifungal activity); the description at page 69, lines 4-6, teaching that enhanced anti-fungal activity of these strain is achieved "by producing greater levels of anti-fungal agent on a cell basis"; the description at page 68, line 12 to page 75, line 17 exemplifying several different mutants of *Pseudomonas* strain AN5 that produce higher concentrations of sugar acids per cell (see in particular page 70, lines 12-15, Table 4 and Table 5).

Claim 25

Claim 25 has been amended to define the deposited *Pseudomonas* strain AN5*rif* (AGAL Accession No. NM00/09624). Support for claim 25 is to be found *inter alia* in original claim 25.

Claim 26

Claim 26 has been amended to define mutants of *Pseudomonas* strain AN5 that are genetically engineered. Support for claim 26 as amended is to be found *inter alia* in the description at page 26, lines 3-21.

Claim 27

Claim 27 has been amended to define those mutants of *Pseudomonas* strain AN5 that are genetically engineered so as to comprise a transposon, cosmid or multi-copy plasmid. Support

for claim 27 is to be found *inter alia* in the description at page 26, line 10; at page 28, lines 6-19; and at page 68, line 25 to page 69, line 17.

Claim 76

Claim 76 has been amended to further define the mutant as having the sugar acid biosynthesis characteristics of the deposited strain AGAL Accession No. NM00/09624. Support for claim 76 is to be found *inter alia* in original claim 76.

Claims 90 and 91

The dependency of claims 90 and 91 has been amended pursuant to the cancellation of claim 88. Consequential amendments are proposed to introduce antecedent basis for the feature of a mutant of *Pseudomonas* strain AN5. Support for claims 90 and 91 is to be found *inter alia* in original claims 90 and 91, respectively.

Claim 100 and 101

The dependency of claims 100 and 101 has been amended pursuant to the cancellation of claim 98. Consequential amendments are proposed to introduce antecedent basis for the feature of a mutant of *Pseudomonas* strain AN5. Support for claims 100 and 101 is to be found *inter alia* in original claims 100 and 101, respectively.

New claims 103, 107 and 111

New claims 103, 107 and 111 further define the mutant of *Pseudomonas* strain AN5 as a naturally-occurring mutant having antibiotic resistance. Support for these new claims is to be found *inter alia* in the description at page 66, lines 4-7; and at page 68, lines 12-23.

New claims 104, 108 and 112

New claims 104, 108 and 112 further define the mutant of *Pseudomonas* strain AN5 as a naturally-occurring mutant having antibiotic resistance that confers on the mutant an ability to grow on rifampicin. Support for these new claims is to be found *inter alia* in the description at page 66, lines 4-7; and at page 68, lines 18-23.

New claims 105 and 109

New claims 105 and 109 further define the biocontrol agent of the base claims as comprising a genetically-engineered mutant of *Pseudomonas* strain AN5 having increased ability to reduce or prevent the growth of a fungus compared to *Pseudomonas* strain AN5 as determined in a standard bioassay for growth of the fungus. Support for newly-added claims 105 and 109 is to be found *inter alia* in the description at page 26, lines 3-21.

New claims 106 and 110

New claims 106 and 110 further define the mutant of *Pseudomonas* strain AN5 as comprising a transposon, cosmid or multi-copy plasmid. Support for new claims 106 and 110 is to be found *inter alia* in the description at page 26, line 10; at page 28, lines 6-19; and at page 68, line 25 to page 69, line 17.

Given the foregoing remarks, Applicants respectfully submit that the instant amendments introduce no new matter for consideration, and request that the amendments be entered. Upon entry of the proposed amendments, claims 24-27, 76-87, 90-97, and 100-112 will be under examination.

Reconsideration of the application as amended is respectfully requested in view of the remarks below.

Rejection under 35 U.S.C. § 112, Second Paragraph

In the Office Action, the Examiner has rejected claims 24-27 and 73-102 as allegedly indefinite.

The Examiner states that claim 24 is rendered indefinite by the phrase "a level comparable," on the basis that the specification does not provide a standard for ascertaining the requisite degree and because it is allegedly uncertain whether "level" is better/worse or lower/higher than the level provided by the presently claimed strain (page 3, lines 1-3). Without conceding the correctness of this allegation, Applicants have amended claim 24 as indicated above, in a manner that obviates the rejection.

The Examiner states that claims 25-27 are indefinite with regard to the phrase "derivatives," because it is uncertain what strains would be "derivatives" as intended, and the characteristics of those derivatives (page 3, lines 17-19). Without conceding the correctness of this allegation, Applicants have amended claims 25-27 in a manner that obviates the rejection.

The Examiner states that claims 74, 88 and 98 are redundant and indefinite for failing to point out further structural elements (page 3, lines 20-21). Without conceding the correctness of this allegation, Applicants have canceled the rejected claims as noted above, thereby obviating the rejection.

The Examiner states that claims 75, 89 and 99 are indefinite with regard to the phrase "PQQ." Without conceding the correctness of this allegation, Applicants have canceled the rejected claims thereby obviating the rejection.

The Examiner is respectfully requested to reconsider and withdraw the rejection in view of the foregoing amendments and remarks.

Rejection under 35 U.S.C. § 112, First Paragraph

The Examiner rejected all of the claims as amended, as allegedly failing to comply with the written description requirement.

The Examiner states that the limitation "having the same or enhanced ability to reduce or prevent the growth of fungus relative to said *Pseudomonas* strain AN5 *rif* (AGAL Accession No. NM00/09624) and to bacterial cells "comparable" to strain AN5 *rif* have no support in the specification as filed (page 4, lines 9-12).

In response to this rejection, Applicants have amended the claims without prejudice or disclaimer, to define a class of mutants of *Pseudomonas* strain AN5 having enhanced biocontrol activity relative to the parent strain, as opposed to having antifungal activity comparable to strain AN5 *rif*. Applicants respectfully submit that the proposed amendment obviates the new matter rejection raised by the Examiner.

With regard to the presently-amended independent claim 24, the specification clearly describes mutants of *Pseudomonas* strain AN5 ". . . with increased sugar acid biosynthetic capacity and/or sugar acid secretion compared to the naturally occurring isolate, including auxotrophic mutants, replication mutants and recombinant strains that have been produced by the insertion of additional genetic material, such as, for example, extrachromosomal plasmids or integrated DNA, or transposable genetic elements" (page 26, lines 3-10). Means for producing mutants of *Pseudomonas* strain AN5 are described in detail at pages 27 and 28 of the specification, and including the use of chemical and physical mutagens, transposon mutagenesis and insertional mutagenesis. Support for the feature of producing more sugar acid per cell than *Pseudomonas* strain AN5 when cultured in the presence of a carbon source comprising an aldose, as contained in claim 24, is provided in the description at page 66, lines 3-14:

> The inventors have isolated naturally-occurring mutants of *Pseudomonas* AN5 bacteria, and genetically engineered new strains of *Pseudomonas* AN5 bacteria, which produce different amounts of sugar acids when grown on aldose substrates. This has been achieved, for example, by culturing the parent strain on different antibiotics, by introducing a multi-copy plasmid with additional anti-fungal genes into *Pseudomonas* strain AN5, or by transposon mutagenesis of *Pseudomonas* strain AN5.....These mutants and derivatives of *Pseudomonas* strain AN5 produce larger clearance zones in agar plate bioassays. These strains have been tested for biological control protection against take-all in controlled environment cabinet trials.

This claimed feature also finds support at page 69, lines 6-8: "by increasing the capacity of a single cell to produce the anti-fungal compound, a more effective biological control strain may be created."

Moreover, the description at page 68, line 12 to page 70, line 17 and Tables 3 and 4 provides adequate written description of mutants of the parent strain AN5 having increased production of sugar acids and increased anti-fungal activity compared to AN5. In particular, the antibiotic-resistant deposited strain AN5*rif* (AGAL Accession No. NM00/09624), the transformed mutant strain AN5-P1 containing a multi-copy plasmid, a genetically-engineered mutant of *Pseudomonas* strain AN5-M1 comprising the cosmid pLAF3, and the transposon mutant strain AN5-T5 produce higher concentrations of sugar acids than the AN5 parent and have increased anti-fungal activity. More specifically, the description at page 68, lines 18-23 teaches that the deposited strain AN5*rif*" has superior biocontrol characteristics against take-all fungus, on both PDA plates and in pot trials, compared to the parent strain." The description at page 70, line 20 through page 75, line 17 (i.e., Examples 10-12) indicates that this strain produces very high levels of sugar acid.

Further, the description bridging pages 68 and 69 points to a genetically-engineered mutant of *Pseudomonas* AN5-M1 containing the vector pLAF3, in respect of which "each individual cell which colonizes the roots may provide superior protection compared to an individual cell of the parent strain, presumably by producing greater levels of anti-fungal agent on a cell basis."

In addition, the description bridging pages 69 and 70 refers to the genetically-modified mutants of *Pseudomonas* strain AN5 designated AN5-P1 and AN5-T5 that "protect significantly

better than the parent strain, *Pseudomonas* strain AN5" (page 69, lines 27-28) and "produce higher concentrations of sugar acids" (page 70, lines 13-14).

The specification also describes several mutants of *Pseudomonas* strain AN5 having *decreased* anti-fungal activity compared to the parent strain. Although they are beyond the scope of the present claims, such mutants confirm the rationale underlying claim 24, i.e., that sugar acids are the anti-fungal agent produced by *Pseudomonas sp.* In particular, the transposon mutants AN5-MN1, AN5-MN2 and AN5-MN3 were shown by the inventors to have no biocontrol activity, to produce no anti-fungal metabolite, and to be deficient in an enzyme that converts glucose to sugar acid, e.g., at page 66, lines 26-30.

In consideration of the foregoing remarks, Applicants respectfully submit that there is adequate support in the specification as filed to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention as presently claimed.

Rejection under 35 U.S.C. § 102/103

The Examiner has rejected claims 24-27, 73-80, 82-84, 86-94 and 96-102 anticipated by Nayudu *et al.* ("Nayudu"), or in the alternative, as being obvious over Nayudu.

With regard to the rejection of independent claim 24 for anticipation, the Examiner alleges that Nayudu discloses several mutants derived from *Pseudomonas* strain AN5 that are capable of utilizing sugar and producing sugar acids at least to the same degree as the parent strain AN5, as well as being capable of producing biocontrol effects against plant fungal pathogens at least to the same degree as the parent strain. Proceeding on this basis, the Examiner concludes that the mutants/derivatives disclosed by Nayudu are reasonably expected to have identical properties as the "biocontrol properties" of the claimed strain AN5 *rif*, because the presently claimed strain is also the mutant/derivative of the same parent strain AN5 as the disclosed mutants/derivatives." Applicants respectfully traverse *vis-à-vis* the amended claims.

Claim 24 is predicated on the teaching in the specification, that sugar acids produced by *Pseudomonas sp.* are required for effective anti-fungal activity and further, the identification of

several mutants of the *Pseudomonas sp.* AN5 having increased biological control activity against fungi as compared to the parent AN5 strain.[1]

In contrast, Nayudu merely catalogs a series of *Pseudomonas sp.* mutants having modified mucoidy, decreased growth rate, decreased glucose utilization, uracil auxotrophy, or an inability to grow on antibiotics (i.e., antibiosis mutants). Applicants respectfully submit that such mutants are entirely different from the mutants having increased sugar acid production and anti-fungal activity relative to the parent strain AN5, as encompassed by amended claim 24.

At most, Nayudu discloses mutants of *Pseudomonas* strain AN5 that have significantly **reduced** biological control protection in pot trials as compared to the parent AN5 strain (see, e.g., Table 1 of Nayudu). In contrast, claim 24 requires a mutant AN5 strain having **increased** anti-fungal activity relative to the parent AN5 strain. There is no mention or suggestion by Nayudu of any strain having increased anti-fungal activity and sugar acid production relative to the parent strain AN5. Indeed, the Examiner has not provided a single example of a mutant described in the citation that is equivalent to Applicants' strain AN5*rif* in terms of biocontrol or sugar acid production. Thus a skilled artisan would conclude that none of the mutant strains disclosed in Nayudu meet the limitations of amended claim 24.

Accordingly, Applicants maintain that claim 24 is not anticipated by Nayudu. claims 25-27, 73-80, 82-84, 86-94 and 96-102, which depend from claim 24 are also not anticipated for at least the same reasons.

As for the Examiner's allegation that independent claim 24 is obvious over Nayudu, this ground for rejection appears to be moot, as none of the AN5 strains disclosed in Nayudu are shown to have increased sugar acid production relative to the AN5 strain and an increased ability to reduce or prevent growth of a fungus compared to the AN5 strain as required by amended claim 24. Moreover, Nayudu, provides no enabling disclosure of the properties of *Pseudomonas sp.* making them suitable as biocontrol agents, in particular the ability to produce a sugar acid when cultured in the presence of a carbon source comprising an aldose, the ability to colonize the infection site of a fungal pathogen of a plant, and the ability to reduce or prevent the growth of a

[1] Claim 24 recites a mutant of *Pseudomonas* strain 25 having the following characteristics: "(i) it produces more sugar acid per cell than *Pseudomonas* AN5 when cultured in the presence of a carbon source comprising an aldose; (ii) it is capable of colonizing the infection site of a fungal pathogen of a plant; and (iii) by virtue of (i) and (ii), it has increased ability to reduce or prevent the growth of a fungus compared to *Pseudomonas* strain AN5 as determined in a standard biosassay for growth of the fungus" (emphasis added).

fungus as determined in a standard bioassay for growth of the fungus. In fact, Nayudu fails to mention or suggest that a sugar acid is the active anti-fungal compound produced by any *Pseudomonas*, let alone Applicants' *Pseudomonas* strains AN5*rif*, AN5-M1, AN5-P1 or AN5-T5.

Thus, Applicants submit that claim 24 as well as claims 25-27, 73-80, 82-84, 86-94 and 96- 102 dependent from it are not obvious over Nayudu.

Rejection under 35 U.S.C. § 103

The Examiner has rejected claims 24-27, 73-80, 82-84, 86-94 and 96-102 as allegedly obvious over Nayudu in view of Dahiya *et al.* ("Dahiya"), Scnider *et al.* ("Scnider"), and U.S. Pat. No. 4,456,684 ("'684").

The Examiner states that Nayudu teaches bacterial cells that are effective against plant pathogens including the fungus *G. graminis,* but does not disclose anti-fungal activity against *Botrytis faba*. However, it is alleged that Dahiya teaches *Pseudomonas* producing pyrrolnitrin and phenazine antibiotics active against fungi including *G. graminis* and *Botrytis fabae,* and that Scnider teaches the efficacy of antibiotics produced by *Pseudomonas* against various plant pathogens that are able to utilize glucose. Further, it is noted that '684 teaches anti-fungal protective amounts of *Pseudomonas* bacterial cells for treating fungal infections of plants. The Examiner further indicates that Scnider and '684 teach the utilization of glucose by *Pseudomonas sp.* Accordingly, the Examiner contends that it would have been obvious at the time the application was filed, to obtain a biocontrol composition comprising *Pseudomonas sp.* for the treatment of fungal infection in a plant.Applicants respectfully traverse this ground for rejection.

Claim 24 contains a mutant of *Pseudomonas* strain AN5 that has both increased acid production and an increased ability to reduce or prevent the growth of a fungus compared to the *Pseudomonas* AN5 strain. In contrast, the only bacterium that Nayudu teaches as having anti-fungal activity is the parent strain AN5. In addition, Nayudu fails to mention or suggest that a sugar acid produced by the strain is an effective anti-fungal compound. Applicants would like to point out that absent the identification of a sugar acid as the anti-fungal compound, it simply is not possible to screen specifically for a class of bacteria having increased sugar acid production

and, as a consequence, increased anti-fungal activity relative to the parent strain AN5, both of which features are required by claim 24.

Applicants respectfully submit that this failing by Nayudu to mention or suggest the importance of sugar acids to anti-fungal activity of bacterial strains is not cured by any of the other citations relied upon by the Examiner. Nor does any of the citations mention any strain that produces more sugar acid than AN5 and has higher anti-fungal activity compared to AN5 as required by claim 24, let alone suggesting the importance of sugar acids as anti-fungal compounds. Accordingly, it would not be possible for a skilled artisan armed with the prior art to produce a class of bacterial strains as contained in amended claim 24, having increased anti-fungal activity relative to the parent strain AN5 that depends on increased sugar acid production. Clearly, there would be no motivation provided to a skilled artisan to even attempt to produce such a class of bacteria, let alone with any expectation of success absent the teachings of Applicants' specification.

The Examiner also appears not to appreciate the significant distinction between antifungal activity conferred by sufficient sugar acid production as claimed on the one hand, and the production of antibiotics generally *vis-à-vis* sugar utilization on the other hand (see, e.g., page 10, lines 1-4). Applicants respectfully submit that these are functionally unrelated and do not suggest equivalence between the respective bacterial strains.

The antibiotics, as disclosed by Dahiya (e.g., pyrrolnitrin and phenazine antibiotics; see Office Action at page 9, lines 10-12) or Scnider, are not the anti-fungal compound produced by *Pseudomonas sp.* in the presently-claimed context (i.e., a sugar acid). Further, glucose utilization and sugar acid production are not the same, or even necessarily correlated. Thus, the mere teaching of modified glucose utilization by the prior art does not suggest that sugar acid production is necessarily increased. Applicants respectfully submit that the disclosure of a bacterium having modified glucose utilization (e.g., as in Nayudu) fails to suggest to a skilled artisan that sugar acid levels in the bacterium will also be modified, or that such sugar acid will be at a level sufficient to confer protection against a fungal pathogen, let. Nor does the prior art suggest that a particular level of sugar acid may be required for any level of anti-fungal activity, let alone a level of anti-fungal activity higher than that of the *Pseudomonas* AN5 strain as required by claim 24.

For the reasons presented herein and those already on record, Applicants maintain that claim 24, as well as claims 25-27, 73-80, 82-84, 86-94 and 96-102 dependent from it, is clearly non-obvious over Nayudu in view of Dahiya, Scnider, and the '684 patent.

The Examiner is respectfully requested to reconsider and withdraw the rejection in view of the foregoing remarks.

CONCLUSION

In consideration of the foregoing amendments and remarks, Applicants respectfully submit that the instant application is now in condition for allowance, which action is earnestly solicited.

Applicants enclose a Request for Continued Examination, with the required fee of $395; a Petition for Three Month Extension of Time, with the required fee of $510; and a $75 check for excess claim fees. Please apply any other charges to deposit account 06-1050, referencing attorney docket 13377-002001.

Respectfully submitted,

Date: 8-10-05

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